Quality analysis of *Polygala tenuifolia* root by ultrahigh performance liquid chromatography-tandem mass spectrometry and gas chromatography-mass spectrometry

Dan Wu, Jirui He, Yueming Jiang, Bao Yang*

Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou, China

* Corresponding author:
Bao Yang, PhD, Professor.
Tel: +86 20 37083042
E-mail: yangbao@scib.ac.cn.

Abstract

Polygala tenuifolia root is usually used for functional food due to attractive health benefits. In this work, ultrahigh performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) and gas chromatography-mass spectrometry (GC-MS) were utilised to characterise the bioactive compounds in *P. tenuifolia* root. The UPLC-MS/MS information revealed 36 bioactive compounds, including oligosaccharide esters, polygalasaponins and polygalaxanthones. GC-MS identified 34 volatile compounds with fatty acids as the main chemicals. The leading compound judged by UPLC-MS/MS was tenuifoliside A, and oleic acid was the leading volatile from GC-MS profiles. All the samples showed similar bioactive compounds compositions, but the level of each compound varied. The results of principal component analysis revealed the principal bioactive compounds with significant level variations between samples. These principal chemicals could be used for quality judgement of *P. tenuifolia* root, instead of measuring the levels of all compositional compounds.

Keywords: Bioactive compound; GC-MS; *Polygala tenuifolia*; UPLC-MS/MS;

1. Introduction

The genus *Polygala* includes herbaceous plants, shrubs and small trees, which are distributed over the world. Some *Polygala* species are used in functional foods and folk medicines against inflammation and disorder of central nervous system [1]. As a widely used medicinal plant, *Polygala tenuifolia* has attracted much attention due to the good pharmaceutical properties against insomnia, neurosis and dementia [2]. Moreover, *P. tenuifolia* root can attenuate cognitive dysfunction and relieve the depressive disorder [3,4]. As cognitive dysfunction and depression are common disorders occurred on people nowadays, the prevention effect of *P. tenuifolia* root has attracted much interest by scientists in functional food field. Many functional food products formulated with *P. tenuifolia* root have been developed.

Natural bioactive compounds, like phenolics and carbohydrates, are responsible for the pharmaceutical activities of medicinal herb [5,6]. It has been reported that oligosaccharides, saponins and xanthones in *P. tenuifolia* root are critical chemicals involved in disease treatment and health benefits [7]. The antidepressant effect is mainly attributed to the occurrence of oligosaccharide esters, like 3,6'-disinapolysucrose. Polygalasaponins possess good capability against cognitive impairments [8]. Xanthones have diverse biological profiles, including anticancer, antihypertension and antioxidation [9]. It is obvious that bioactive compounds composition determines the quality of *P. tenuifolia* root. More than two hundred chemicals have been identified from *Polygala* genus. However, the chemical

composition of *P. tenuifolia* root is still not clear. Determination of phytochemical profile by ultrahigh performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) and gas chromatography-mass spectrometry (GC-MS) is a good choice to understand the chemistry nature of medicinal plant.

Metabolomics is being increasingly utilized to gain insight into the chemical composition of biological materials [10]. Two types of analytical techniques, based on MS and nuclear magnetic resonnance spectroscopy (NMR), are widely applied in metabolomics. The MS technique shows better precision and resolution than NMR. Therefore, in this work, the bioactive compounds profile of *P. tenuifolia* root was analysed by both UPLC-MS/MS and GC-MS. It is useful to judge the quality of commercial *P. tenuifolia* root.

2. Materials and Methods

2.1. Plant material

Four *P. tenuifolia* roots were collected from Sanmenxia of Henan province (1 and 2#) and Yuncheng of Shanxi province (3 and 4#), respectively. Each sample was pulverized into powder and screened through 60-mesh sieve.

2.2. UPLC-MS/MS analysis

Two grams of *P. tenuifolia* root powder were extracted with 20 ml of methanol for one week in the dark at room temperature. The extract was centrifuged at 8000*g* for 10 min. Each sample was extracted in triplicate. The supernatant was collected and subjected to UPLC-MS/MS analysis. Acquity^{TM} UPLC equipped with Acquity^{TM} UPLC BEH C18 column (1.7 μm, 2.1×150 mm) was used for chemicals isolation. A gradient elution programme was conducted as follows: 0-45 min, from 5% to 50% solvent B; 45-48 min, from 50% to 100% solvent B; 48-51 min, from 100% to 5% solvent B; 51-53 min, 5% solvent B. The flow rate was 0.25 ml/min. Solvent A was 0.1% formic acid in water, and solvent B was acetonitrile. Detection was performed on a triple quadrupole mass spectrometer using an electrospray ionization (ESI) interface. Ionization of the analytes was achieved using ESI in negative mode. The interface conditions were as follows: capillary voltage, 1.0 KV; cone voltage, 10 eV; collision voltage, 10 eV; source temperature, 150 ºC; desolvation temperature, 500 ºC; cone gas, nitrogen at a flow rate of 50 L/h; desolvation gas, nitrogen at a flow rate of 800 L/h; collision gas, argon at a flow rate of 0.2 mL/min. Mass scan was set in the range of *m*/*z* 50-2000. The daughter ion mode was monitored at a collision voltage of 10-40 eV.

2.3. GC-MS analysis

Two grams of *P. tenuifolia* root powder were extracted with 20 ml of hexane/acetone (7:3, v/v) for one week in dark at room temperature. The extracts

were centrifuged at 8000*g* for 15 min. The supernatants were collected and subjected to trimethylsilyl derivatization due to the occurrence of fatty acids. The trimethylsilyl derivatization was conducted as the method of Yang et al. [11]. A gas chromatography/mass spectrometer (GCMS-QP 2010, Shimadzu, Kyoto, Japan) was used to analyse the chemical composition. The derivatives were loaded into a RTX-5 capillary column. The temperature program was set as follows: the initial temperature of column was 50 ^{o}C, holding for 1 min, increasing to 250 ^{o}C at 3 ^{o}C/min, holding for 17 min, increasing to 280 ^{o}C at 10 ^{o}C/min; injection temperature: 250 ^{o}C. The ion source of mass spectrometer was set at 250 ^{o}C. The scanning *m*/*z* range was 20-550 amu. 1 μl of sample was injected and the split ratio was 10:1. The carrier gas was helium with a flow rate of 1.0 ml/min. The peaks were identified by NIST database and retention index.

2.4. Data analysis

For each sample, the peak heights of metabolites in mass spectra were recorded and averaged over three replicates. An unsupervised multivariate statisitcal method, principal component analysis (PCA), was used on UV-scaling data by software SIMCA-P version 11.5 (Umetrics, Umeå, Sweden). PCA was performed to visualize the clustering of different samples without any knowledge of their group membership.

3. Results

3.1. Semi-polar metabolites composition identified by UPLC-MS/MS

The methanolic extract of *P. tenuifolia* root was analysed by UPLC-MS/MS to identify the semi-polar metabolites. Figure 1 shows the full scan ESI-MS spectra in negative mode. The identified chemicals of main peaks are listed in Table 1. Those metabolites with relatively high peak were interpreted here. The leading peak was tenuifoliside A (Retention time 19.79 min, Fig. 2). The MS spectra had an $[M-H]^-$ at *m/z* 680.7 and $[2M-H]^-$ at *m/z* 1362.7. The fragment ions were interpreted as follows: *m/z* 442.6 (loss of trimethoxycinnamoyl and hydroxyl group), 281.0 (loss of trimethoxycinnamoyl and fructose), 238.9 (breaking of glucose), 136.7 (*p*-hydroxy benzoic acid). Peak at 17.16 min gave an $[M-H]^-$ and $[2M-H]^-$ at *m/z* 752.8 and 1506.9, which was identified as sibiricose A4. Its fragment ion at *m/z* 223.1 indicated synapic acid, and *m/z* 546.7 was due to the loss of a synapoyl group. Peak at 22.36 min gave an $[M-H]^-$ and $[2M-H]^-$ at *m/z* 766.8 and 1535.8, respectively. The fragment ions at *m/z* 528.8 (loss of sinapoyl and hydroxyl), 237.3 (trimethoxy cinnamic acid) and 222.6 (synapic acid) further confirmed this peak as tenuifoliside C. A peak at 12.96 min was polygalaxanthone XI (MW 568), a xanthone C-glycoside, which gave an $[M-H]^-$ and $[2M-H]^-$ at *m/z* 567.0 and 1134.5, respectively. The fragment ions at *m/z* 435.1 (loss of apiose), 416.9 (loss of apiose and H_2O) and 344.8 (breaking of glucose at 0,3 bonds) further confirmed this structure. 1,2,3,7-Tetramethoxyxanthone (MW 316) was detected at 47.40 min with $[M-H]^-$ at *m/z* 314.9, and fragment ions at *m/z* 214.4 (loss of $C_5H_9O_2$) and 116.8 (loss of

$C_{10}H_{14}O_4$). The chemical structure of above compounds are shown in Fig. 2. Moreover, some other chemicals, including polygalasaponins, xanthones and sucrose esters, were also identified, which could be found in literatures [12-15].

3.2. Volatile metabolites composition identified by GC-MS

The volatile metabolites were extracted by hexane/acetone, trimethylsilylated and determined by GC-MS, which gave 34 volatile compounds, including alchol, carboxylic acids, esters, and terpenoids (Table 2). The leading volatile chemical was oleic acid in all the samples tested. Other volatile compounds with percentage higher than 1% were 2,5-dihydroxy-3,6-bis(hydroxymethyl)-1,4-dioxane, hexadecanoic acid, 9-octadecenoic acid methyl ester, 9,12-octadecadienoic acid, stearic acid, 11-eicosenoic acid, eicosanoic acid and eicosanoic acid 2-glycerol ester.

3.3. Principal component analyses of *P. tenuifolia* root based on their metabolites profiles

Principal component analysis was conducted on methanolic extract of *P. tenuifolia* root samples. The first two principal components explained 75.9% of the total variance, in which the first principal component explained 45.8% of the total variance and the second principal component did 30.1%. The loading plot (Fig. 3A) revealed the main compounds responsible for the sample difference. The first component was represented by arillalose B, sibiricoses A1, A6, tenuifolisides B, C,

arillanin A, 6-O-benzoyl 3'-O-(4-hydroxy-3,5-dimethoxy-*E*- cinnamoyl) acylsucroses, tenuifolioses A, E, L, polygalasaponin XLV and reinioside B, which were characterised as saponins and oligosaccharide esters. The second component was mainly defined by 1-hydroxy-2,3-methylenedioxyxanthone, sibiricose A2, tenuifolioses B, F and G, which were characterised as xanthone and oligosaccharide esters. Score plot showed the statistical similarity between samples. 1# *P. tenuifolia* root was characterized by large amounts of arillalose B, sibiricose A2 and telephiose E, while 3# root showed high levels of tenuifoliose B and I. They were located in two opposite quadrants in the scroe plot which indicated significant differences. 2# root was located in the proximity of 1# root in the score plot.

Figure 3B shows the results of principal component analysis on volatile metabolites levels. The first two principal components explained 81.5% of the total variance, 51.9% by the first component and 29.6% by the second component. Major metabolites defining the first component were propanedioic acid, 2-hydroxyl benzoic acid, hexadecanoic acid, palmitelaidic acid, 9-octadecenoic acid methyl ester, stearic acid, 11-eicosenoic acid, eicosanoic acid, tetracosanoic acid and eicosanoic acid 2-glycerol ester. The second component was mainly characterized by 5-ethyl-*m*-xylene, 1,2,3,4-tetramethyl-benzene and 2,3-dihydroxyl-propanoic acid. 1# *P. tenuifolia* root was characterized by high levels of 5-ethyl-*m*-xylene and 1,2,3,4-tetramethyl-benzene. 3# root was characterized by high level of 2,3-dihydroxyl-propanoic acid.

4. Discussion

4.1. Phytochemical profile of *P. tenuifolia* root

Phytochemical analysis supplies a good way to evaluate the quality of commercial biological resources, like medicinal herbs and fruits. At present, LC-MS and GC-MS are two efficient and precise techniques to resolve the phytochemical profile of plant resources [16,17]. The former technique can identify semi-polar metabolites with advantages of high precision and short time consumed. It is widely applied to the characterization of plant secondary metabolites. GC-MS can reveal the chemistry nature of volatile metabolites [18], which define the flavor of plant [19]. GC-MS also provides complementary information to LC-MS analysis to obtain a complete phytochemical profile. From the bioactive compounds profile obtained in this work, it could be concluded that four samples had similar metabolites profiles, but level of each metabolite varied.

More than seventy metabolites were identified from *P. tenuifolia* root in this work. Three sucrose esters, including sibiricose A4, tenuifoliside A and C, were detected as the leading metabolites in the UPLC-MS/MS profile. They were reported as the bioactive compounds responsible for the pharmaceutical effect of *P. tenuifolia* root against depression [20]. Several saponins and xanthones were also detected as important constituents, including 1,2,3,7-tetramethoxyxanthone, 1-hydroxyl-2,3-methylenedioxyxanthone, polygalaxanthones III, V and VIII, as well as

polygalasaponins XLV and XXXV. Polygalasaponins play a critical role in the characteristic bioactivities of *P. tenuifolia* root, like neuroprotection and memory improvement. Cognitive dysfunction is a symptom of central nervous system disorder, which endangers the life quality of people. Xue et al. have pointed out that polygalasaponins can significantly prevent scopolamine-induced cognitive impairments in mice [8]. Even though the numbers and levels of saponins and xanthones in the UPLC-MS profile were less than oligosaccharide esters (Table 1 and Figure 1), they works as prerequisites to the health-beneficial effects of *P. tenuifolia* root. GC-MS analysis showed that the major volatile compounds were fatty acids, which were in consistent with the determinations of Wang et al. [21]. Amongst, oleic acid was the leading volatile metabolite. It is also a pharmacologically active chemical. Though the contribution of volatile compounds to the pharmaceutical activities of *P. tenuifolia* root is much less than non-volatile compounds, they influences the flavor characteristics to some extent.

Based on the level and importance to the pharmacological functions, oligosaccharide esters, polygalasaponins and xanthones identified in the UPLC-MS profile could be selected to evaluate the quality of *P. tenuifolia* root. A *P. tenuifolia* root with relatively high levels of bioactive compounds is considered as having good quality. Through ANOVA analysis of representative metabolites levels (data not shown), including tenuifoliside A, tenuifoliose A, sibiricose A4 and onjisaponin W, the results indicated that four *P. tenuifolia* roots had no significance of differences on

the levels of tenuifoliside A, tenuifoliose A and onjisaponin W. For sibiricose A4, 2# sample showed significantly ($p < 0.05$) higher level than 1#, but no significant ($p > 0.05$) differences between 2#, 3# and 4# were observed. The results showed that 1# sample had a worse quality comparing with other three samples.

4.2. Multivariate statistical analysis of *P. tenuifolia* root metabolites

PCA is an unsupervised clustering technique of identifying patterns in data, expressing the data in a way to emphasize their similarities and differences. Through reducing the number of dimensions, it can define a limited number of principal components which describe independent variation in the results [10,22]. In this work, PCA was carried out on semi-polar and volatile metabolites of *P. tenuifolia* root to find principal variables defining the pharmacological quality. In the loading plot, it displays the influence of individual spectral peaks in each principal component and describes the separation shown in the score plot [23]. The points in the loading plot far away from zero point represent characteristic markers with most confidence to each group. Figure 3A shows the characteristic compounds for each sample. The first two principal components explained most of the total variance. The first principal components were labeled by tenuifolioses (A, E and L) and polygalasaponin XLV. The second principal component was labeled by tenuifolioses (B, F and G) and 1-hydroxy-2,3-methylene-dioxyxanthone. It was surprised that tenuifoliside A had the leading peak in the UPLC-MS profile. However, it was not the representive chemical

in the two first pricinpal components due to the insignificant variance. Only those metabolites having significant variance will be calculated in the PCA model. Tenuifolioses are the characteristic oligosaccharide esters in *P. tenuifolia* root [24], which are constructed by pentasaccharide, phenolic acid and acetyl moieties. It is very rare to find these oligosaccharides in other plant resources. And they shows good neuroprotective and antioxidant activities, which make *P. tenuifolia* root a good choice of traditional folk medicine. Seven xanthones were detected in the UPLC-MS profile, and only 1-hydroxy-2,3-methylenedioxyxanthone was included in the principal component. Xanthones show good potential in the treatment against inflammation, asthma and allergy [1]. They also exhibits bioactivity against neroblastoma [25]. Polygalaxanthones are a type of xanthones characterized by C-glycoside moiety, which contribute partially to the bioactivity of *P. tenuifolia* root. Through PCA calculation, some of oligosaccharide esters, xanthones and saponins were selected as principal components. These metabolites can be chosen to determine the quality of commercial *P. tenuifolia* roots, instead of measuring the levels of all metabolites. This result also proved that UPLC-MS/MS and GC-MS were efficient for quality evaluation of medicinal plants [26].

5. Conclusions

Analysis of bioactive compounds profile was an efficient way to define the

quality of *P. tenuifolia* root. By using UPLC-MS/MS and GC-MS, the semi-polar and volatile metabolites were identified and their levels were statistically compared. Tenuifoliside A was the leading metabolite in *P. tenuifolia* root. Xanthones and saponins are another two important types of metabolites occurred in *P. tenuifolia* root. PCA plot indicated the representive metabolites for each sample, and these metabolites could be utilized to judge the commercial quality of *P. tenuifolia* root.

6 Acknowledgements

We are grateful to the financial support from Guangdong Natural Science Fundation for Distinguished Young Scholar (No. S2013050014131), Youth Innovation Promotion Association of Chinese Academy of Sciences, Pearl River Science and Technology New Star Fund of Guangzhou (No. 2014J2200081), Guangdong Natural Science Foundation (No. S2011020001156) and International Foundation for Science (No. F/4451-2).

References

[1] El Sayah M, Cechinel V, Pinheiro TR, et al. *In vitro* effect of the extract and the 1,7-dihydroxy-2,3-dimethoxy xanthone from *Polygala cyparissias* on the contractions induced by inflammatory mediators and ovalbumin in normal and actively sensitised trachea from guinea pig. Inflamm. Res. 1999;48:218-223.

[2] Park HJ, Lee K, He H, et al. Effects of *Polygala tenuifolia* root extract on proliferation of neural stem cells in the hippocampal CA1 region. Phytother. Res. 2008;22:1324-1329.

[3] Hu Y, Liao HB, Liu P, et al. A bioactive compound from *Polygala tenuifolia* regulates efficiency of chronic stress on hypothalamic-pituitary-adrenal axis. Pharmazie 2009;64:605-608.

[4] Ikeya Y, Takeda S, Tunakawa M, et al. Cognitive improving and cerebral protective effects of acylated oligosaccharides in *Polygala tenuifolia*. Biol. Pharm. Bull. 2004;27:1081-1085.

[5] Yang B, Chen F, Hua YL, et al. Prooxidant activities of quercetin, p-courmaric acid and their derivatives analysed by quantitative structure-activity relationship. Food Chem. 2012;131:508-512.

[6] Zhu QQ, Jiang YM, Lin S, et al. Structural identification of (1→6)-α-D-glucan, a key responsible for the health benefits of longan, and evaluation of anticancer activity. Biomacromolecules 2013;14:1999-2003.

[7] Klein LC, de Andrade SF, Cechinel V. A pharmacognostic approach to the *Polygala* genus: Phytochemical and pharmacological aspects. Chem. Biodivers. 2012;9:181-209.

[8] Xue W, Hu JF, Yuan YH, et al. Polygalasaponin XXXII from *Polygala tenuifolia* root improves hippocampal-dependent learning and memory. Acta Pharm. Sinic. 2009:30:1211-1219.

[9] Na Y. Recent cancer drug development with xanthone structures. J. Pharm. Pharmacol. 2009;61:707-712.

[10] Bernillon S, Biais B, Deborde C, et al. Metabolomic and elemental profiling of melon fruit quality as affected by genotype and environment. Metabolomics 2013;9:57-77.

[11] Yang B, Jiang YM, Zhao MM, et al. Structural characterisation of polysaccharides purified from longan (*Dimocarpus longan* Lour.) fruit pericarp. Food Chem. 2009;115:609-614.

[12] Dao TT, Dang TT, Nguyen PH, et al. Xanthones from *Polygala karensium* inhibit neuraminidases from influenza A viruses. Bioorg. Med. Chem. Lett. 2012;22:3688-3692.

[13] Fu J, Zuo L, Yang JZ, et al. Oligosaccharide polyester and triterpenoid saponins from the roots of *Polygala japonica*. Phytochemistry 2008;69:1617-1624.

[14] Zhang DM, Miyase T, Kuroyanagi M, et al. Polygalasaponins XLII-XLVI from roots of *Polygala glomerata*. Phytochemistry 1998;47:459-466.

[15] Ling Y, Li ZX, Chen MC, et al. Analysis and detection of the chemical constituents of Radix polygalae and their metabolites in rats after oral administration by ultrahigh-performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry. J. Pharmaceut. Biomed. 2013;85:1-13.

[16] Mari A, Lyon D, Fragner L, et al. Phytochemical composition of *Potentilla*

anserina L. analyzed by an integrative GC-MS and LC-MS metabolomics platform. Metabolomics 2013;9:599-607.

[17] Chen RC, Wei KJ, Wang TM, et al. Simultaneous quantification of antibiotic dyes in aquatic products and feeds by liquid chromatography-tandem mass spectrometry. J. Food Drug Anal. 2013; 21:339-346.

[18] Chen HC, Peng LW, Sheu MJ, et al. Effects of hot water treatment effects on the essential oils of calamondin. J. Food Drug Anal. 2013;21:363-368.

[19] Besada C, Sanchez G, Salvador A, et al. Volatile compounds associated to the loss of astringency in persimmon fruit revealed by untargeted GC-MS analysis. Metabolomics 2013;9:157-172.

[20] Hu Y, Liao HB, Dai-Hong G, et al. Antidepressant-like effects of 3,6-disinapoyl sucrose on hippocampal neuronal plasticity and neurotrophic signal pathway in chronically mild stressed rats. Neurochem. Int. 2010;56:461-465.

[21] Wang Y, Chang L, Zhao X, et al. Gas chromatography-mass spectrometry analysis on compounds in volatile oils extracted from Yuan Zhi (*Radix Polygalae*) and Shi Chang Pu (*Acorus Tatarinowii*) by supercritical CO_2. J. Trad. Chinese Med. 2012;32:459-464.

[22] Park MK, Cho IH, Lee S, et al. Metabolite profiling of Cheonggukjang, a fermented soybean paste, during fermentation by gas chromatography-mass spectrometry and principal component analysis. Food Chem. 2010;122:1313-1319.

[23] Yuk J, McIntyre KL, Fischer C, et al. Distinguishing Ontario ginseng landraces and ginseng species using NMR-based metabolomics. Anal. Bioanal. Chem. 2013;405:4499-4509.

[24] Miyase T, Iwata Y, Ueno A. Tenuifolioses A-F, oligosaccharide multi-esters from the roots of *Polygala-tenuifolia* Willd. Chem. Pharm. Bull. 1991;39:3082-3084.

[25] Mak NK, Li WK, Zhang M, et al. Effects of euxanthone on neuronal differentiation. Life Sci. 2000;66:347-354.

[26] Yang B, Yang HS, Chen F, Hua YL, Jiang YM. Phytochemical analyses of *Ziziphus jujuba* Mill. var. *spinosa* seed by ultrahigh performance liquid chromatography-tandem mass spectrometry and gas chromatography-mass spectrometry. Analyst 2013;138:6881-6888.

Table 1. Semi-polar metabolites occurred in the methanolic extract of *P. tenuifolia* root measured by UPLC-MS/MS.

No.	Retention time (min)	MW	$[M-H]^-$ (m/z)	$[2M-H]^-$ (m/z)	Fragment ions (m/z)	Chemicals
1	5.29	462	460.9	922.9	299.1, 136.9	Sibiricose A3
2	7.5	518	516.7	1034.9	340.6, 192.8	Arillalose B
3	7.96	548	546.8	1094.8	222.6, 204.9	Sibiricose A6/A1
4	10.11	548	546.9	1094.3	222.8, 204.8	Sibiricose A6/A1
5	10.96	568	566.8		435.2, 314.5	Polygalaxanthone III or VIII
6	11.73	538	536.7		386.7, 266.7	Sibiricaxanthone B
7	12.75	568	566.5		434.8, 314.9	Polygalaxanthone III or VIII
8	12.96	568	567.0	1134.5	435.1, 416.9, 344.8	Polygalaxanthone XI
9	13.16	562	560.7	1123.2	399.0, 341.0, 236.8	Sibiricose A2
10	13.69	256	255.0	511.0	212.9, 168.5	1-Hydroxy-2,3-methylenedioxyxanthone
11	13.77	550	548.8		467.3, 254.9	-
12	14.3	668	666.7	1334.9	461.1, 280.8, 239.1	Tenuifoliside B
13	14.4	754	752.7	1506.7	705.2, 528.5, 205.0	-
14	16.3	582	580.9		549.0, 323.0, 272.7	Polygalaxanthone V
15	16.45	754	752.9	1507.1	705.0, 692.5, 630.8	Telephiose E
16	17.16	754	752.8	1506.9	546.7, 223.1	Sibiricose A4
17	17.71	724	722.7	1447.3	696.5, 546.8, 516.6	Arillanin A
18	19.21	652	650.7		528.8, 222.5, 204.5	Acylsucroses 3'-O-(4-hydroxy-3,5-dimethoxy-E-cinnamoyl), 6-O-benzoyl

19	19.56	1484	1482.8			Tenuifoliose G[a]
20	19.79	682	680.7	1362.7	442.6, 281.0, 238.9, 136.7	Tenuifoliside A
21	21.85	1526	1525.4		688.7	Tenuifoliose F[a]
22	21.9	1496	1494.9			Tenuifoliose L[a]
23	22.36	768	766.8	1535.8	528.8, 237.3, 222.6	Tenuifoliside C
24	22.87	1660	1659.1			Polygalasaponin XLV[a]
25	23.12	1296	1294.8			Tenuifoliose C[a]
26	23.19	738	736.9	1477.5	704.8	Reiniose A[a]
27	23.94	1308	1306.8		1160.8, 163.3, 145.0	Tenuifoliose I
28	24.45	1338	1336.9		894.5, 320.6	Senegose L
29	24.69	1296	1294.9		1118.5, 881.4	Senegose K
30	25.48	1308	1306.8		964.7	Reinioside B[a]
31	26.07	1338	1336.8			Tenuifoliose B[a]
32	26.38	1350	1348.9		1308.4, 1250.1	Polygalasaponin XXXV
33	26.47	1526	1525.2			Tenuifoliose F[a]
34	26.99	1380	1379.0		1336.6, 390.8	Tenuifoliose A
35	39.91	1732	1731.2		1587.8, 425.4	Onjisaponin W
36	47.40	316	314.9		214.4, 116.8	1,2,3,7-Tetramethoxyxanthone

a, the possible structure; -, unknown.

Table 2. Volatile metabolites occurred in hexane/acetone extract of *P. tenuifolia* root determined by GC-MS.

No.	Retention time (min)	Chemicals
1	5.86	isobutonol
2	6.55	2-ethoxyethyl acetate
3	9.10	2,2',5,5'-tetrahydro-2,2'-bifuran
4	10.95	octyl-3-ol
5	11.44	phenol
6	12.02	propanedioic acid
7	12.29	4-oxo-pentanoic acid
8	12.71	o-cymene
9	13.00	benzenamine
10	13.98	5-ethyl-*m*-xylene
11	14.16	1,2,3,4-tetramethyl-benzene
12	15.19	2,3-dihydroxyl-propanoic acid
13	22.11	2-benzyl-1,3-dioxolane
14	24.72	decanoic acid
15	31.08	2-hydroxyl benzoic acid
16	34.89	2,3-dimethyl-3-hydroxyglutaric acid
17	38.35	9H-carbazole
18	41.56	undecanedioic acid
19	42.58	cinoxate
20	43.89	2,5-dihydroxy-3,6-bis(hydroxymethyl)-1,4-dioxane
21	48.41	palmitelaidic acid
22	49.29	hexadecanoic acid

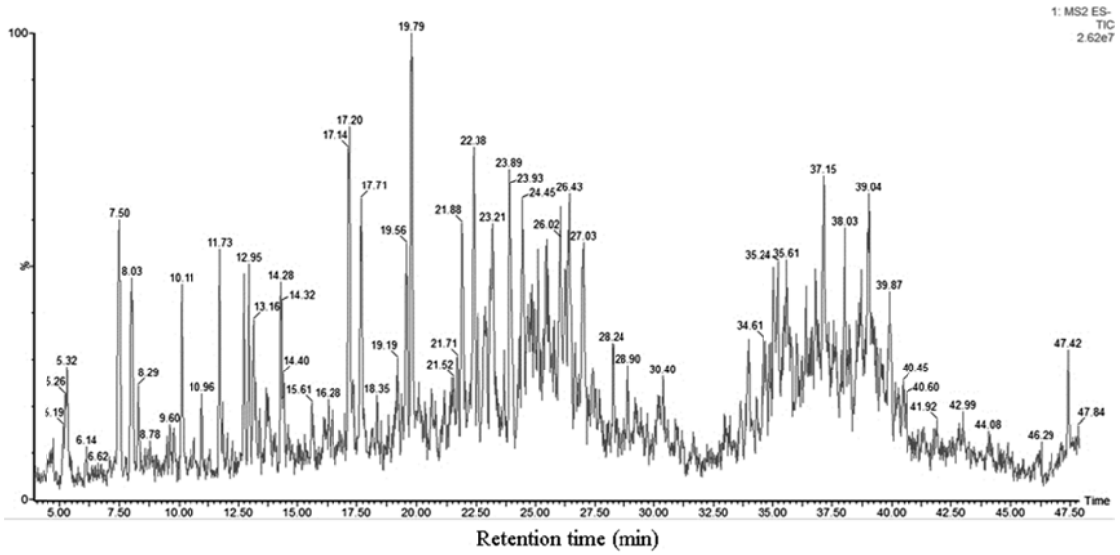
23	50.87	9-octadecenoic acid, methyl ester
24	54.34	9,12-octadecadienoic acid
25	54.52	oleic acid
26	55.06	stearic acid
27	59.62	11-eicosenoic acid
28	60.29	eicosanoic acid
29	63.12	isooctyl phthalate
30	65.22	tetracosanoic acid
31	66.68	thymol-glucoside
32	68.38	eicosanoic acid,2-glycerol ester
33	69.82	squalene
34	89.42	22,23-dibromostigmasterol acetate

Figure legends

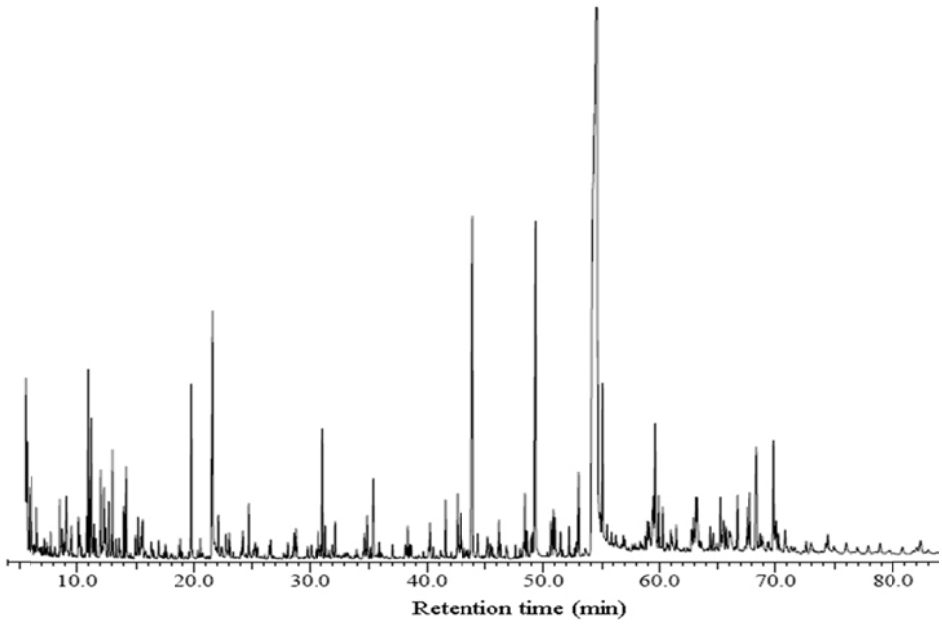
Figure 1. UPLC-MS and GC-MS profiles of *P. tenuifolia* root. A, total ion chromatogram analysed by UPLC-MS; B, total ion chromatogram analysed by GC-MS.

Figure 2. Structures of characteristic metabolites in *P. tenuifolia* root.

Figure 3. Plots of PCA on semi-polar and volatile metabolites of *P. tenuifolia* root. Loading plot (A) and score plot (A) are the results of semi-polar metabolites; Loading plot (B) and score plot (B) are the results of volatile metabolites.

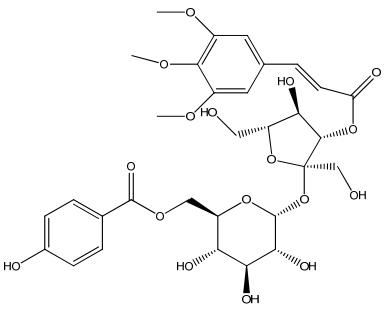


A

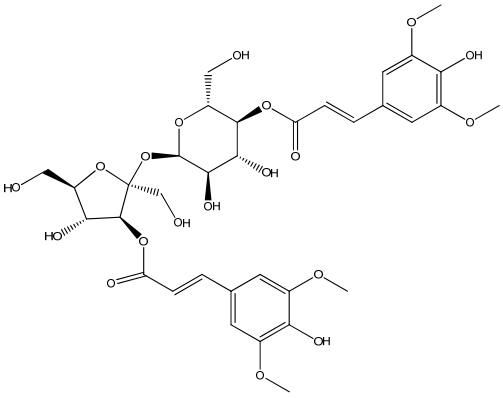


B

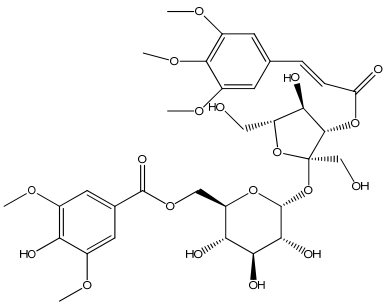
Figure 1



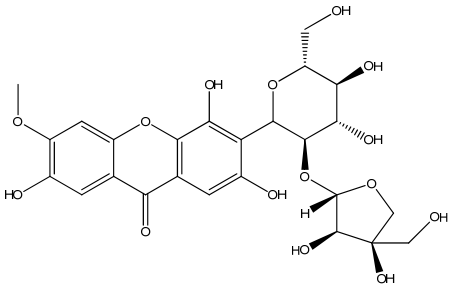
Tenuifoliside A



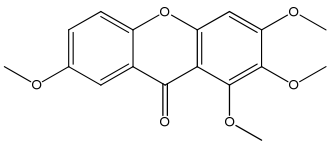
Sibiricose A4



Tenuifoliside C



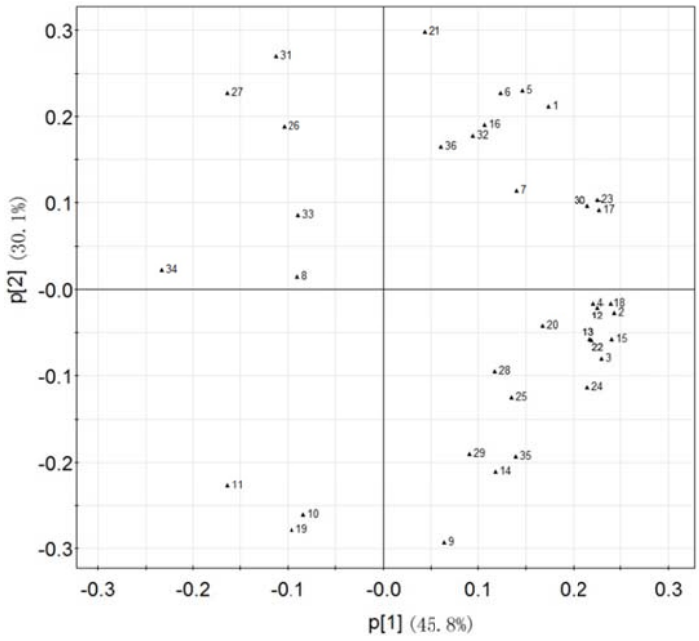
Polygalaxanthone XI



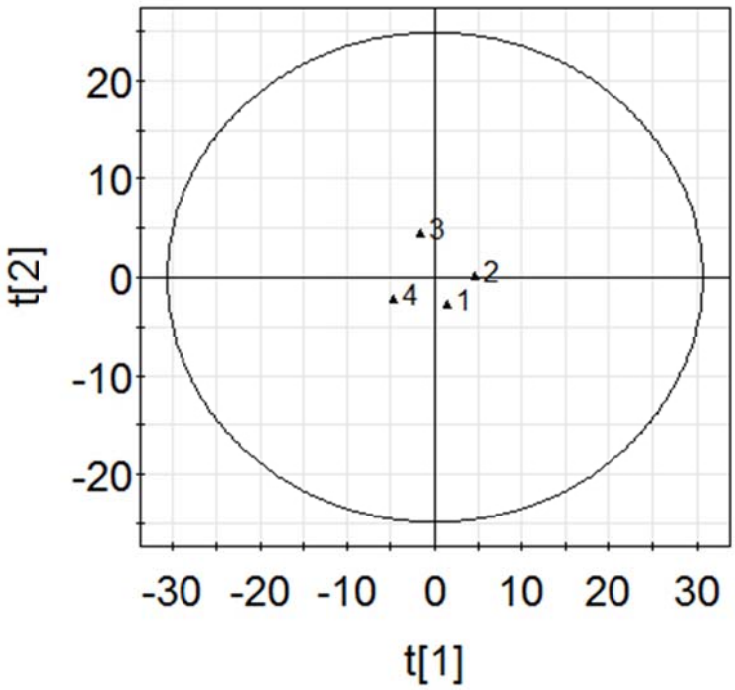
1,2,3,7-Tetramethoxyxanthone

Figure 2

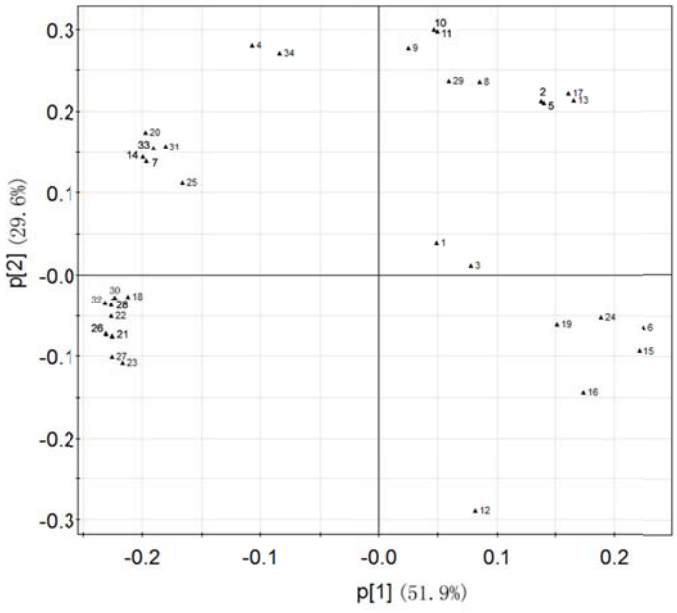
Figure 3



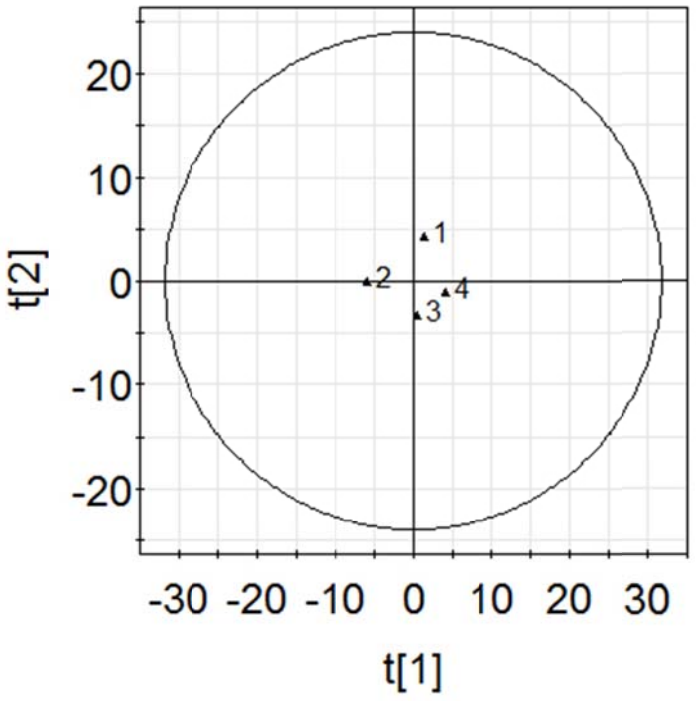
Loading plot (A)



Score plot (A)



Loading plot (B)



Score plot (B)